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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-5
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6
Appendices.....	6

INTRODUCTION

The intracellular polyamines, spermidine, spermine, and putrescine, play an important role in the proliferation and death of normal and malignant cells. As a consequence, work has focused on development of inhibitors of this metabolic pathway. The initial purpose of the ongoing studies is to evaluate the therapeutic and preventive actions of one polyamine analog, DENSPm (N¹, N¹¹-diethylnorspermine or (bis) ethylnorspermine) in breast cancer. This will be accomplished through four related technical objectives. They include: 1) to test the therapeutic efficacy of DENSPm against human breast cancer cell lines in nude mice, 2) to use transient organ cultures of normal and malignant human breast tissues to assess effects of DENSPm on biological and pharmacological parameters relevant to antineoplastic activity, 3) to evaluate the efficacy of DENSPm in a phase II trial in women with metastatic breast cancer, and 4) to evaluate the chemopreventive activity of DENSPm in the rat mammary tumor model.

BODY

Technical Objective 1: To test therapeutic efficacy of DENSPm against estrogen receptor-positive MCF-7 and estrogen receptor-negative MDA-MB-468 human breast cancer cell lines growing in nude mice and assess tumors for possible biological or pharmacological end-points which predict subsequent tumor response.

As noted last year, the initial animal studies proposed were delayed because of the move of the Cancer Center faculty and the animal facilities to the new Bunting-Blaustein cancer research building in January, 2000. Animal studies were begun and then aborted because of concern about possible contamination of cell lines. These were cleared and a set of studies with MDA-MB-468 cells was begun. Growth of tumors was extremely slow, making it impossible to carry out the therapy studies proposed in a timely fashion. As a consequence, a decision was made to use the estrogen receptor-negative MDA-MB-231 cells in place of the MDA-MB-468 cells. During that time, a series of new polyamine analogs became available to us through a collaboration with the pharmaceutical company, Slil. These are of great interest in preclinical studies and have the advantage of a corporate backer interested in their clinical development. Thus animal studies are now in progress using the MDA-MB-231 cells and several promising Slil compounds. Three different Slil compounds are now being tested at two doses each. A treatment schedule of intraperitoneal treatment daily for 5 days out of 21 days was chosen; a total of three cycles will be administered. Animals have just completed the first cycle of therapy without apparent toxicity. Weekly tumor measurement is ongoing. Results of these studies will be used to determine how to extend the work in this technical objective as well as technical objectives 2 and 4.

Technical Objective 2: To use transient organ culture of normal and malignant human breast tissues to assess the effects of DENSPm on biological and pharmacological parameters relevant to antineoplastic activity as identified in Technical Objective 1.

In these studies normal and malignant breast tissues obtained from discarded mastectomy specimens are incubated with or without 10 uM DENSPm for 24 hours in a transient organ culture system. Tissues are then harvested and fixed for subsequent immunohistochemical studies for SSAT. Sample accrual continues; it was interrupted briefly on two occasions over the last year. First, the operating rooms and pathology suite were moved to the new Weinberg Cancer Building in September, 2000. At the same time, the laboratory of Dr. Gabrielson moved from the Johns Hopkins Bayview campus to a new location adjacent to the Weinberg and Bunting-Blaustein Buildings on the main Hopkins campus. During that time of clinical and laboratory transition, these studies were interrupted to focus on establishment of the new facilities. Collection and treatment then resumed only to be stopped briefly in the summer of 2001 because of the OHRP ban on federally-funded research activities at John Hopkins. The tissue acquisition

protocol has since been re-approved in an expedited fashion. These studies will continue over the last year of the grant. In addition, extension to other compounds such as the Slil compounds mentioned above will be considered.

Technical Objective 3: To evaluate the efficacy of DENSpm in a phase II trial in women with metastatic breast cancer.

A total of 16 patients have enrolled on protocol J9951 (An Open-Label, Single Center, Phase 2 Study of Intravenous Diethylnorspermine DENSPM in the Treatment of Patients with Previously Treated Metastatic Breast Cancer) since April, 2000. All patients had evidence of progressive metastatic breast cancer and had been treated with at least one but no more than two prior palliative chemotherapy regimens. Each cycle of therapy consists of a 15-minute infusion of DENSpm (100 mg/m² daily X 5) given every 3 weeks. The primary study objective is to estimate if at least 20% of patients are progression-free after 4 months. The overall accrual goal is 34 patients (30 evaluable) with a two-stage design. The second stage of accrual will proceed if two or more patients among the first 15 evaluable patients are progression-free at 4 months. This study is currently closed to active accrual because of the OHRP actions noted above. We are actively assessing the eligibility and clinical outcomes of the first 16 patients enrolled. Upon preliminary review, it appears that all patients are eligible and that no patient remained progression-free after four months of treatment. Toxicity was minimal. Final assessment of patient records is in progress at present. If these findings remain accurate, then the trial will be formally closed as the guidelines for early closure will have been met. It should be noted that this study was submitted to the Hopkins IRB to permit treatment and follow-up of enrolled patients after the OHRP closure and such approval has been granted. An abstract reporting our findings in this trial was accepted for poster presentation at the 24th Annual San Antonio Breast Cancer Symposium in December, 2001 and a copy of the abstract is included in this progress report. Finally, based on discussions with the sponsor of the trial, GelTex, it is likely that clinical development of DENSpm will not be pursued further by that company. This supports our decision to move our preclinical development studies to other compounds such as those whose development is supported by Slil as they are more likely to move into further clinical testing.

Technical Objective 4: To evaluate the chemopreventive activity of DENSpm in the DMBA rat mammary tumor model.

Implementation of this technical objective has not yet been undertaken as results from Technical Objective 1 are needed to guide the design of these studies. It is expected that these studies will begin in year 3; however, it is likely that they will evaluate the effects of one or more the Slil compounds for all of the reasons outlined above.

KEY RESEARCH ACCOMPLISHMENTS

- Completion of a phase II clinical trial to assess the efficacy of DENSpm in women with advanced breast cancer.
- Successful implementation of a plan to collect polyamine analog-treated and untreated transient organ cultures harvested from tumor tissues from discarded mastectomy specimens.
- Commencement of xenograft studies to evaluate the antineoplastic effects of a new generation of polyamine analogs

REPORTABLE OUTCOMES

Presentations and publications:

The phase II study performed as the central part of this grant will be presented as a poster at the 24th Annual San Antonio Breast Cancer Symposium in December, 2001 and the abstract is in press in the proceedings for the meeting in Breast Cancer Research and Treatment.

Grants awarded:

Related studies to pursue preclinical and clinical activity of other polyamine analogs in breast cancer were funded as part of Project 3C (Robert Casero, Ph.D. , PI and Nancy Davidson, MD co-investigator) in the Johns Hopkins Breast Cancer SPORE grant funded on September 30, 2000 (NIH CA88843). These NCI-funded studies do not include the Slil compounds mentioned here.

CONCLUSIONS

A series of laboratory and clinical studies to evaluate the effects of a polyamine analog, diethylnorspermine (DENSpm), on growth of human breast cancer cells in nude mouse models, transient organ culture derived from human breast cancer tissues, and women with advanced breast cancer has been undertaken. It is likely that the phase II clinical trial has been completed. Because of the lack of activity of DENSpm in this setting and loss of a corporate sponsor, the preclinical studies have shifted to evaluate the effects of the next generation of polyamine analogs. These studies continue to address the promise of these agents for the treatment and prevention of breast cancer in preclinical and clinical models.

REFERENCES

None

APPENDICES

Abstract entitled "A phase II study of diethylnorspermine (DENSPM) in previously treated patients with metastatic breast cancer (MBC). Antonio C. Wolff, M. Katherine Bowling, Carol DeClue, Deborah K Armstrong, John Fetting, Robert Casero, Jr., and Nancy E. Davidson. Breast Cancer Res Treat (in press).

24th Annual San Antonio Breast Cancer Symposium (Dec 2001) - Abstract Submission #550629

A phase II study of diethylnorspermine (DENSPM) in previously treated patients with metastatic breast cancer (MBC).

Antonio C. Wolff, M. Katherine Bowling, Carol DeClue, Deborah K. Armstrong, John H. Fetting, Robert A. Casero, Jr., Nancy E. Davidson, The Johns Hopkins Oncology Center, Baltimore, MD

Polyamines are ubiquitous intracellular polycationic molecules essential for cell growth and differentiation. Eflornithine (DFMO) irreversibly inhibits ornithine decarboxylase (ODC), the first enzyme in the polyamine biosynthetic pathway, but causes reversible ototoxicity. Several polyamine analogues downregulate ODC while inducing the catabolic enzyme spermidine/spermine N1-acetyltransferase (SSAT). These analogues do not substitute for the natural polyamines, and ultimately cause depletion of intracellular pools and cell growth inhibition. The analogue N1,N11-diethylnorspermine (DENSPM) significantly inhibits the growth of multiple breast cancer cell lines, irrespective of hormone receptor status. A phase I study at Johns Hopkins identified gastrointestinal symptoms (abdominal pain with CT scan changes suggestive of small bowel inflammation) as the dose-limiting toxicity using a 15'-minute infusion days 1-5 repeated every 21 days (Ettinger *et al.*, Proc ASCO 1998). Toxicities seen with other administration schedules (e.g., twice-daily infusions) include paresthesias, neuromotor deficit, and transient creatinine elevation. We present interim results of a follow-up study, an open-label single-center phase II study of DENSPM in pts. with MBC. Pts. were treated with the maximum tolerated dose of 100 mg/m² IV days 1-5 repeated every 21 days based on our previous phase I study. The primary study objective is to estimate if at least 20% of pts. are progression free at 4 months. The overall accrual goal is 34 pts. (30 evaluable) with a 2-stage design. The second stage of accrual will proceed if 2 or more among the first 15 evaluable pts. are progression-free at 4 months. To date, 16 pts (median age 52, range 34-65; PS 1, range 0-1) received 39 cycles of DENSPM (median 2, range 1-4). As expected, no hematologic toxicity was detected. All pts. developed transient perioral paresthesias during drug infusion. There were five episodes of gr. 1 abdominal pain toxicity. All pts received 5-HT3 antagonists; none had nausea > gr. 1. One pt with Cy 2 gr. 3 toxicity (CT changes showing jejunal wall thickening) was taken off study. There were no other gr. 3 treatment-related toxicities. Thus far, best response observed is stable disease. Preliminary results indicate that this dose/schedule is quite tolerable. Initial assessment of efficacy will occur upon completion of the first stage of accrual.